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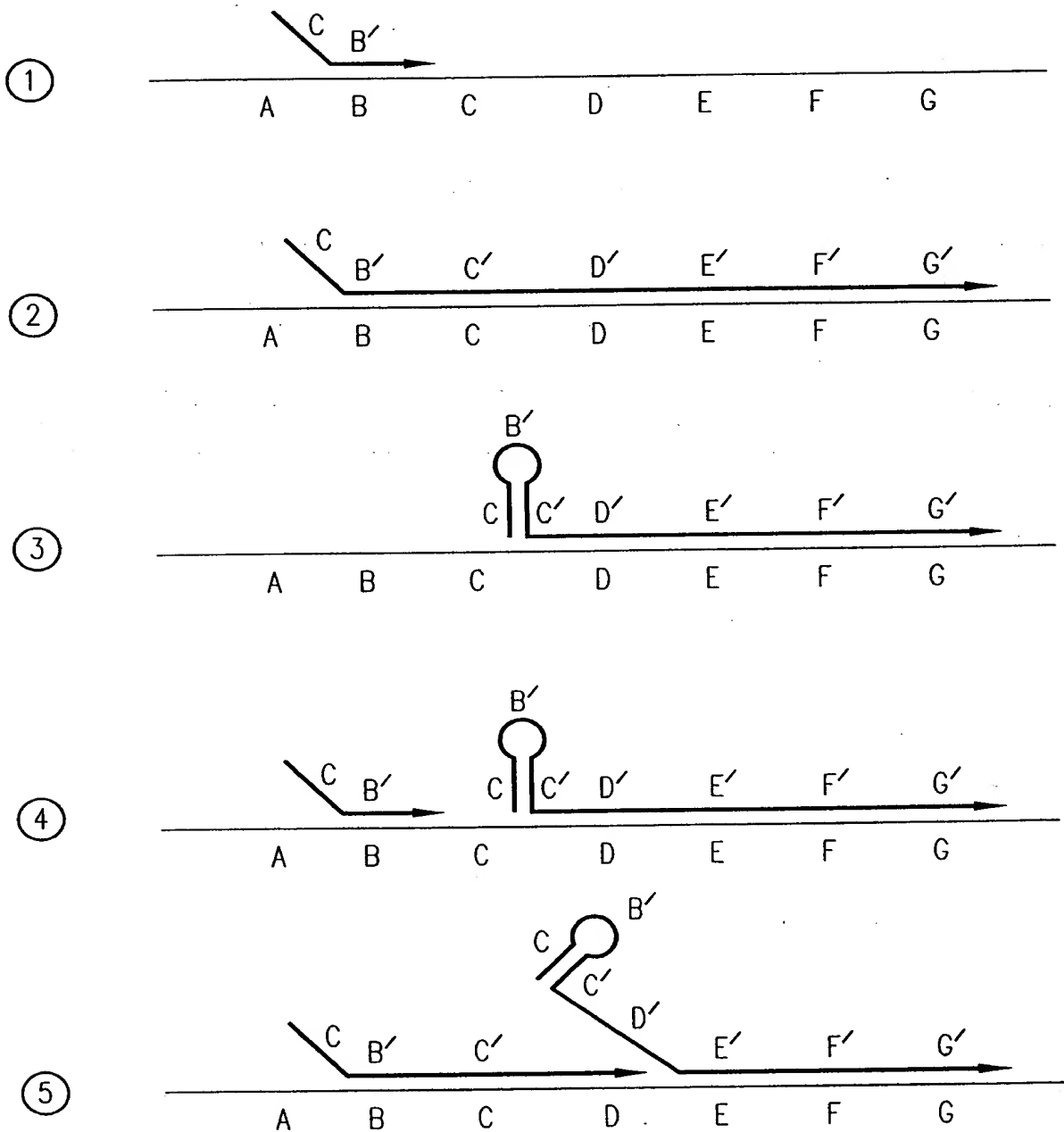


FIG. 1

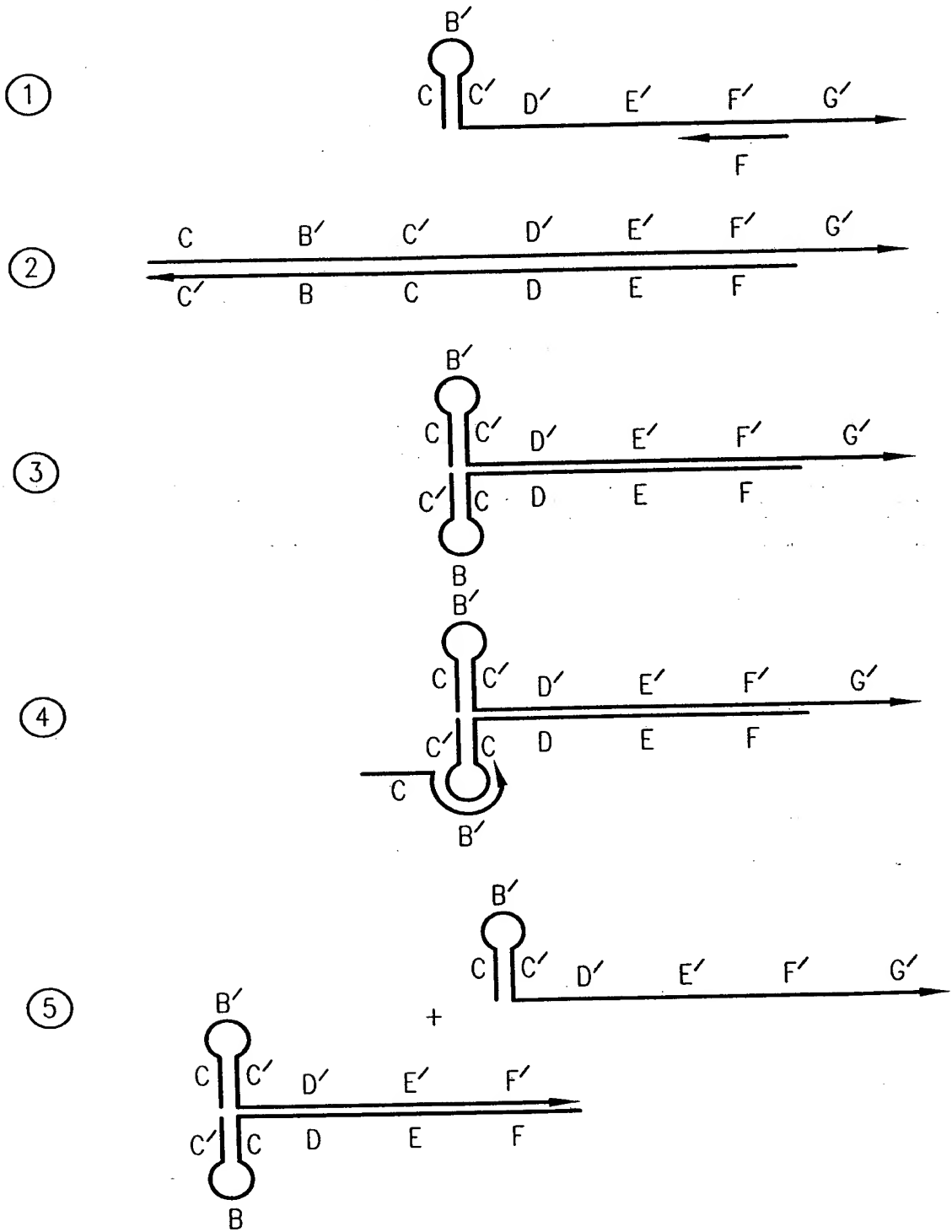


FIG. 2

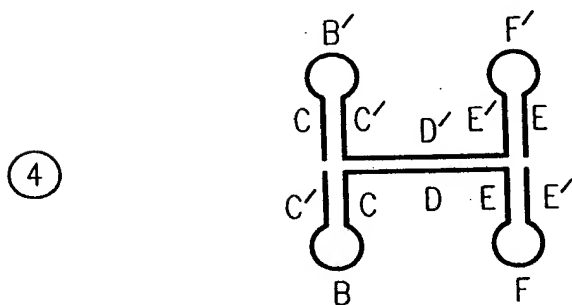
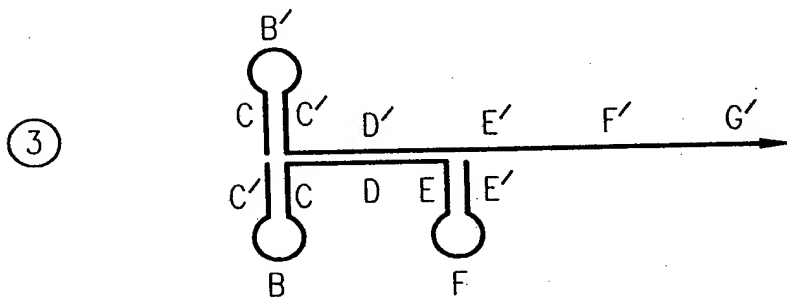
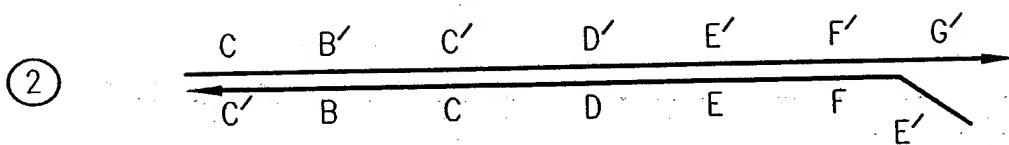
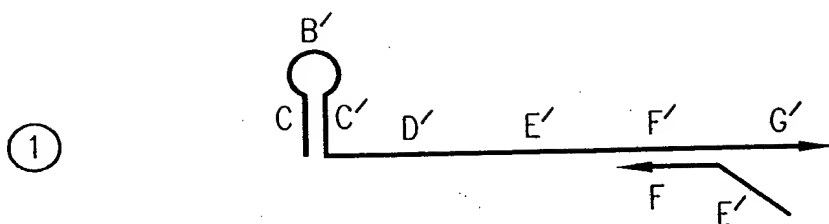
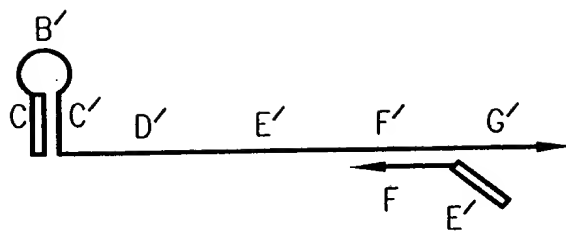


FIG. 3

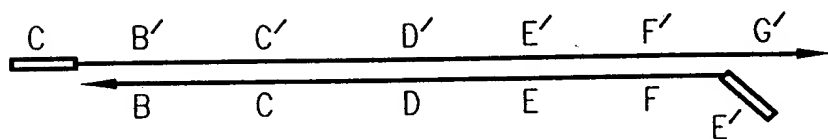


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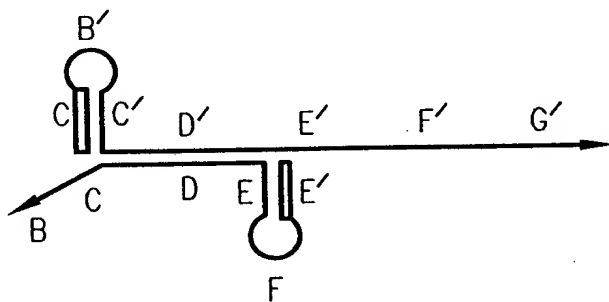
①



②



③



④

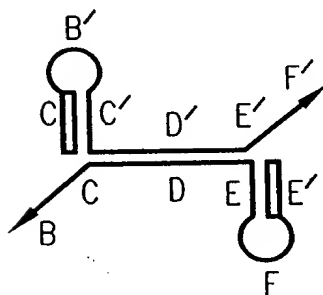


FIG. 4



5/30

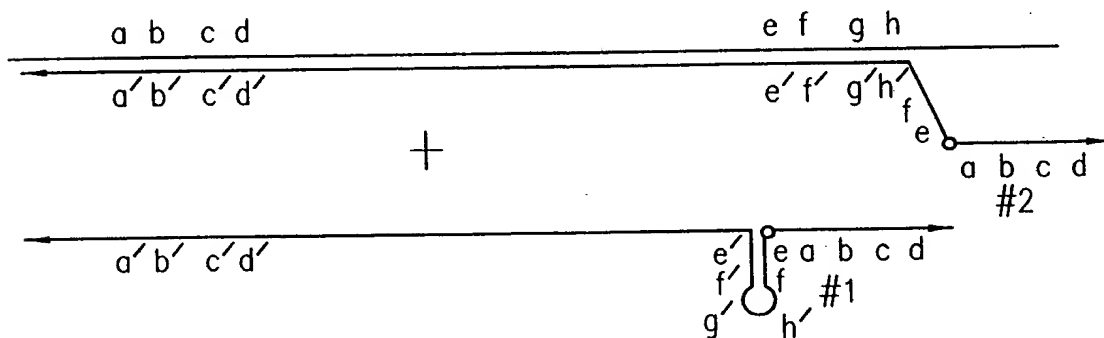
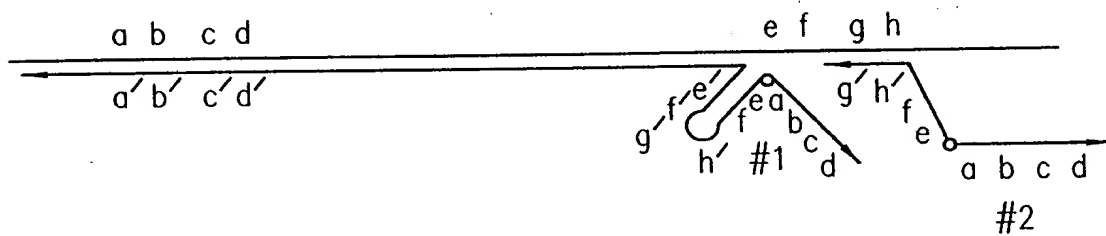
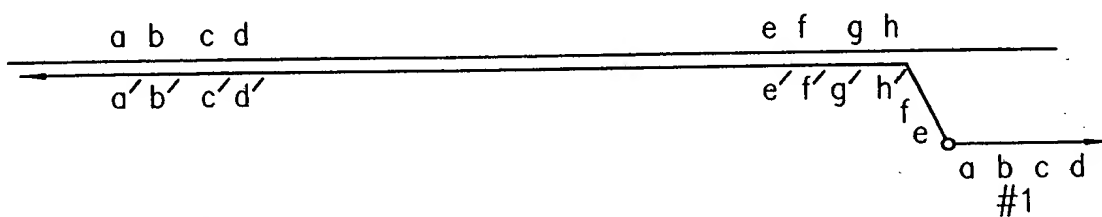
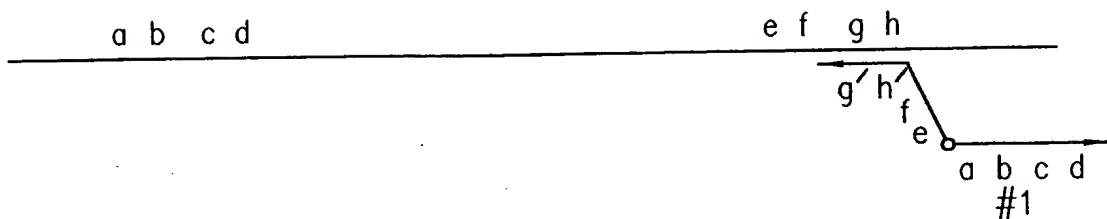


FIG. 5

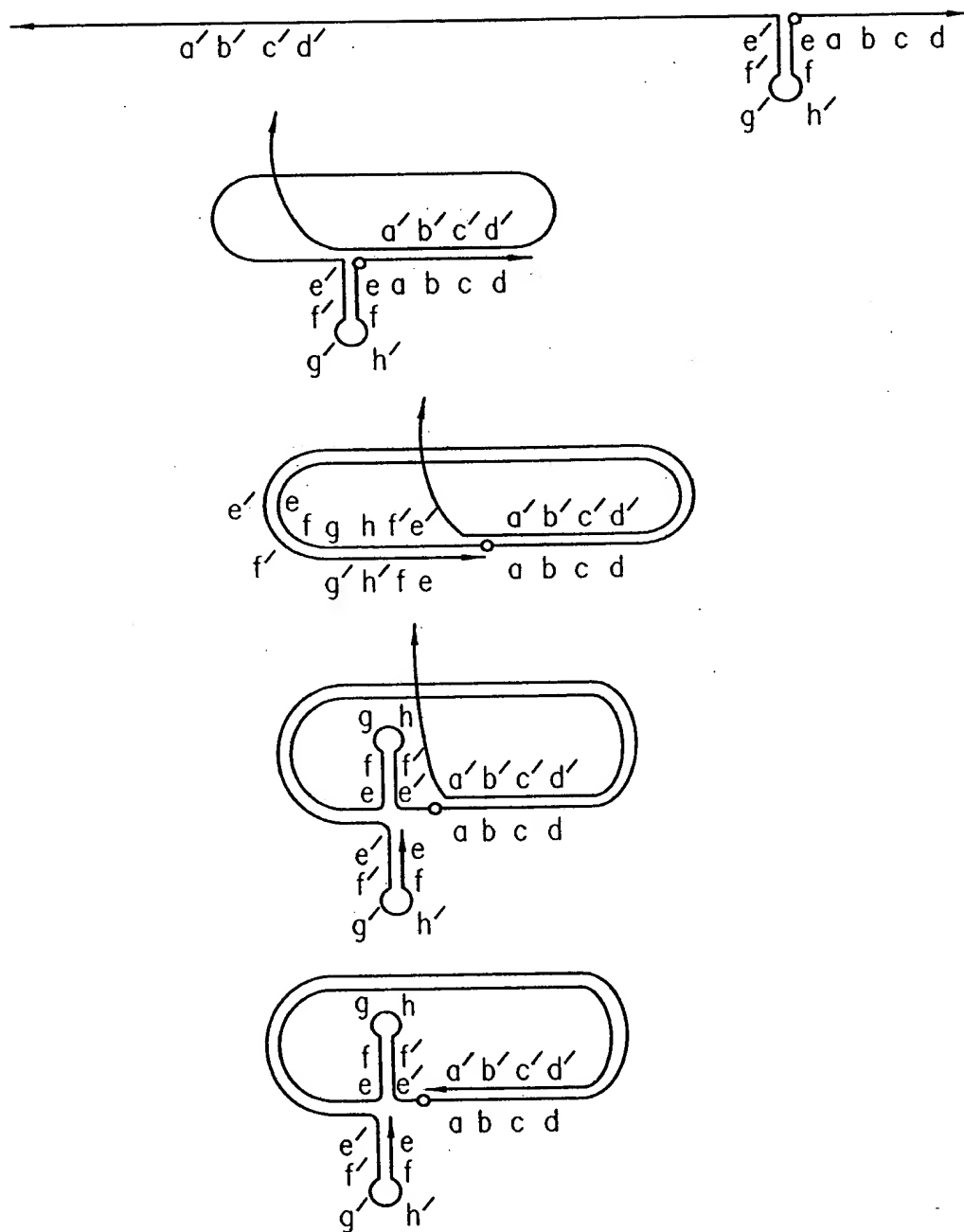


FIG. 6

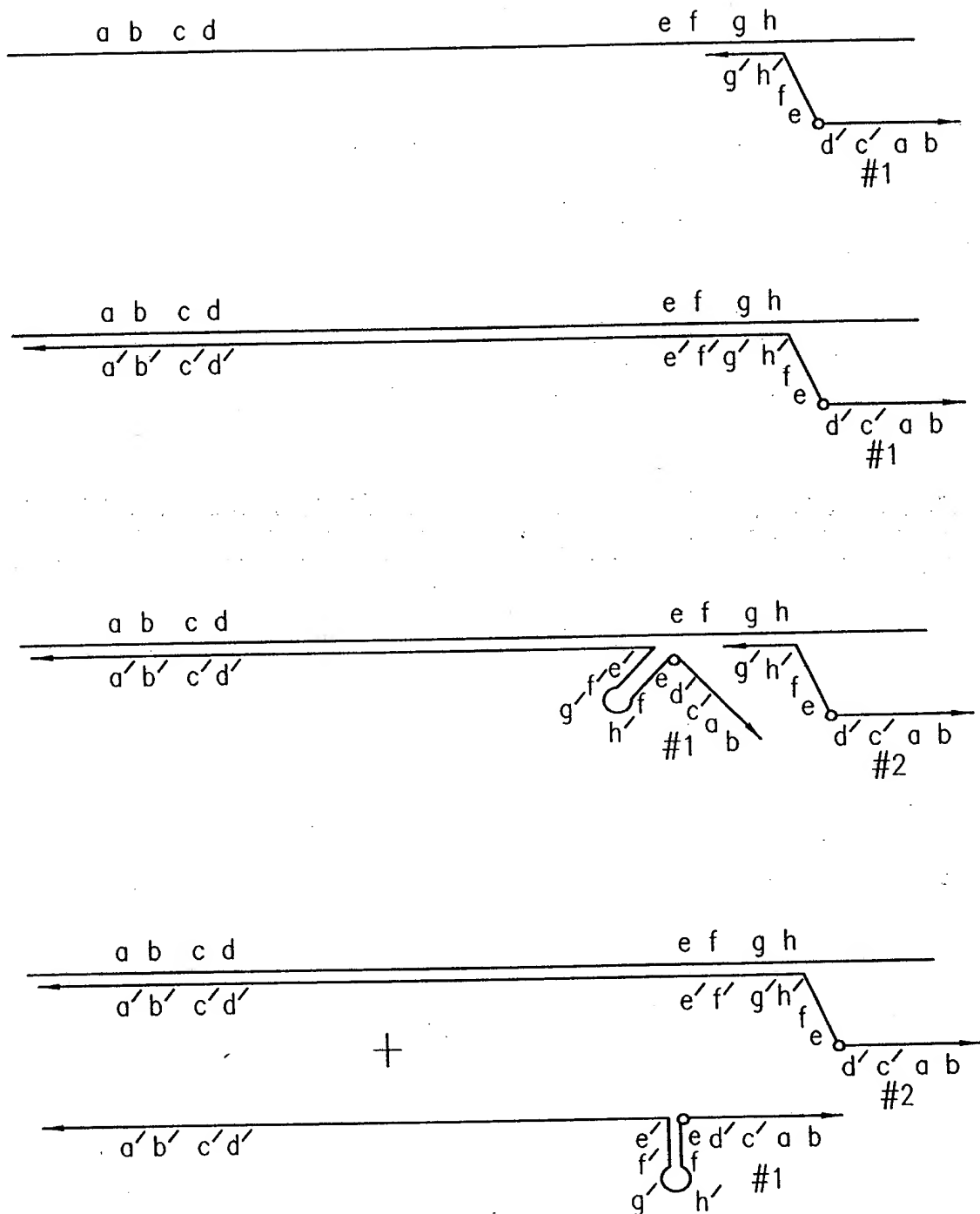


FIG. 7

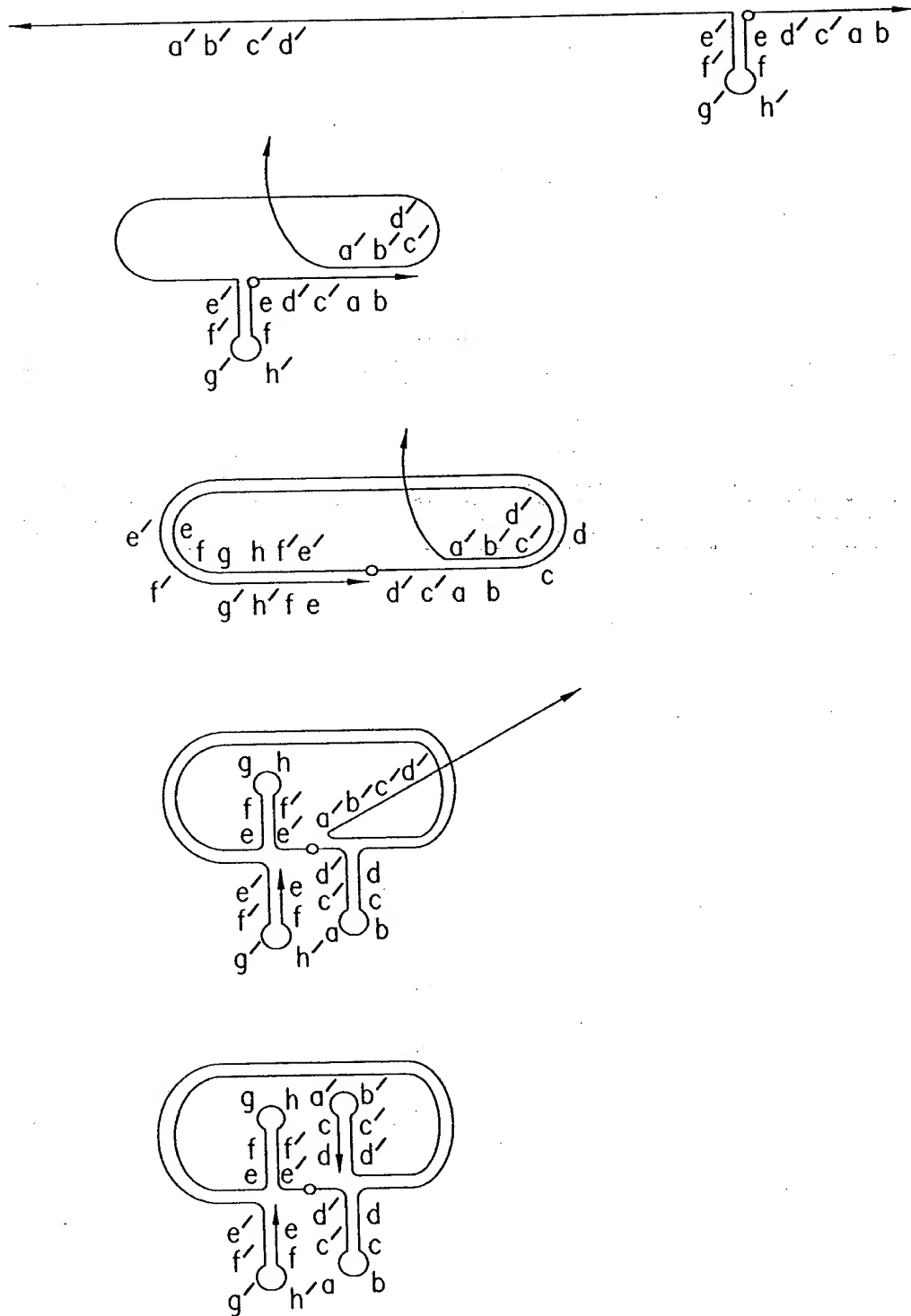


FIG. 8

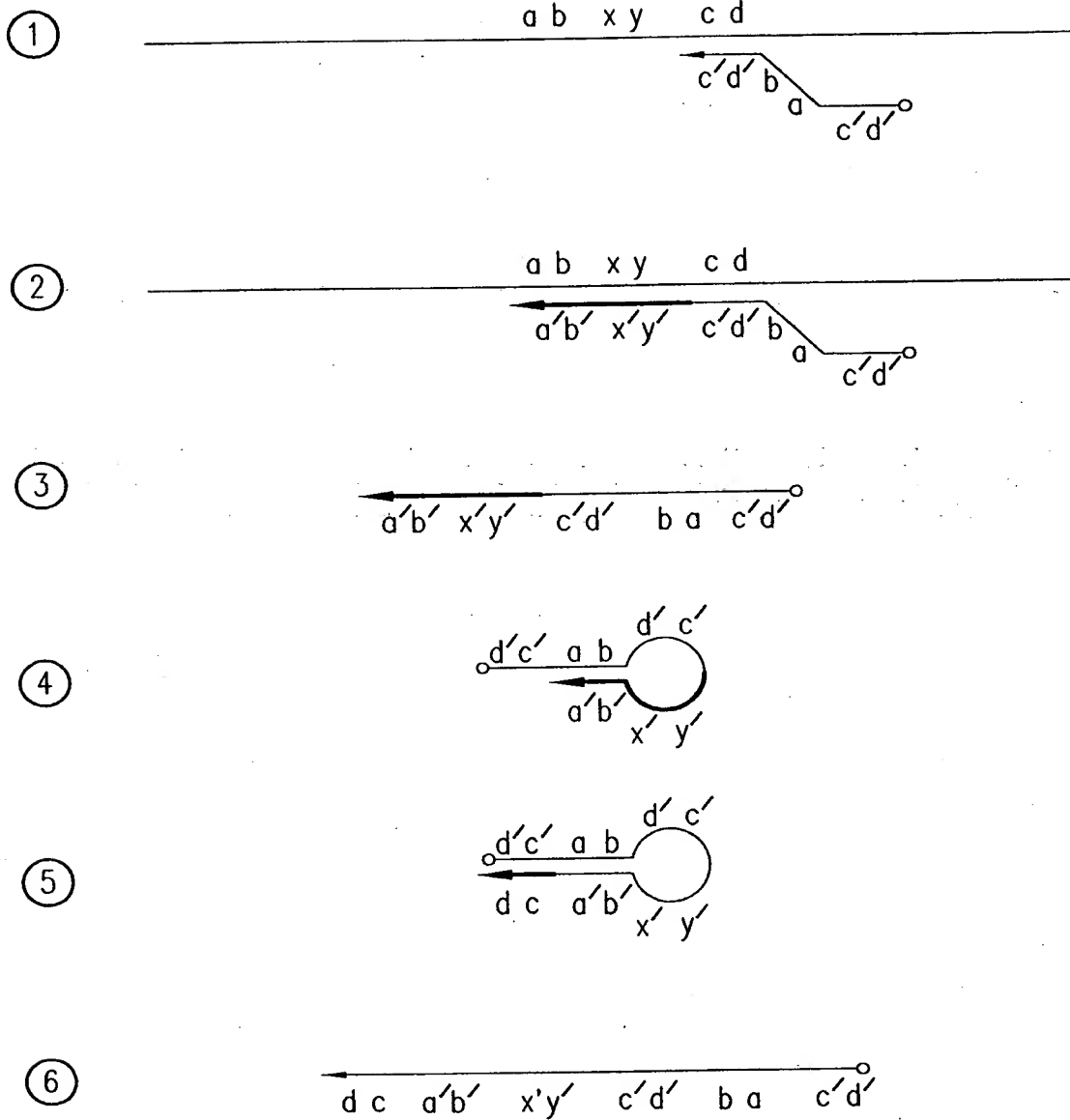


FIG. 9

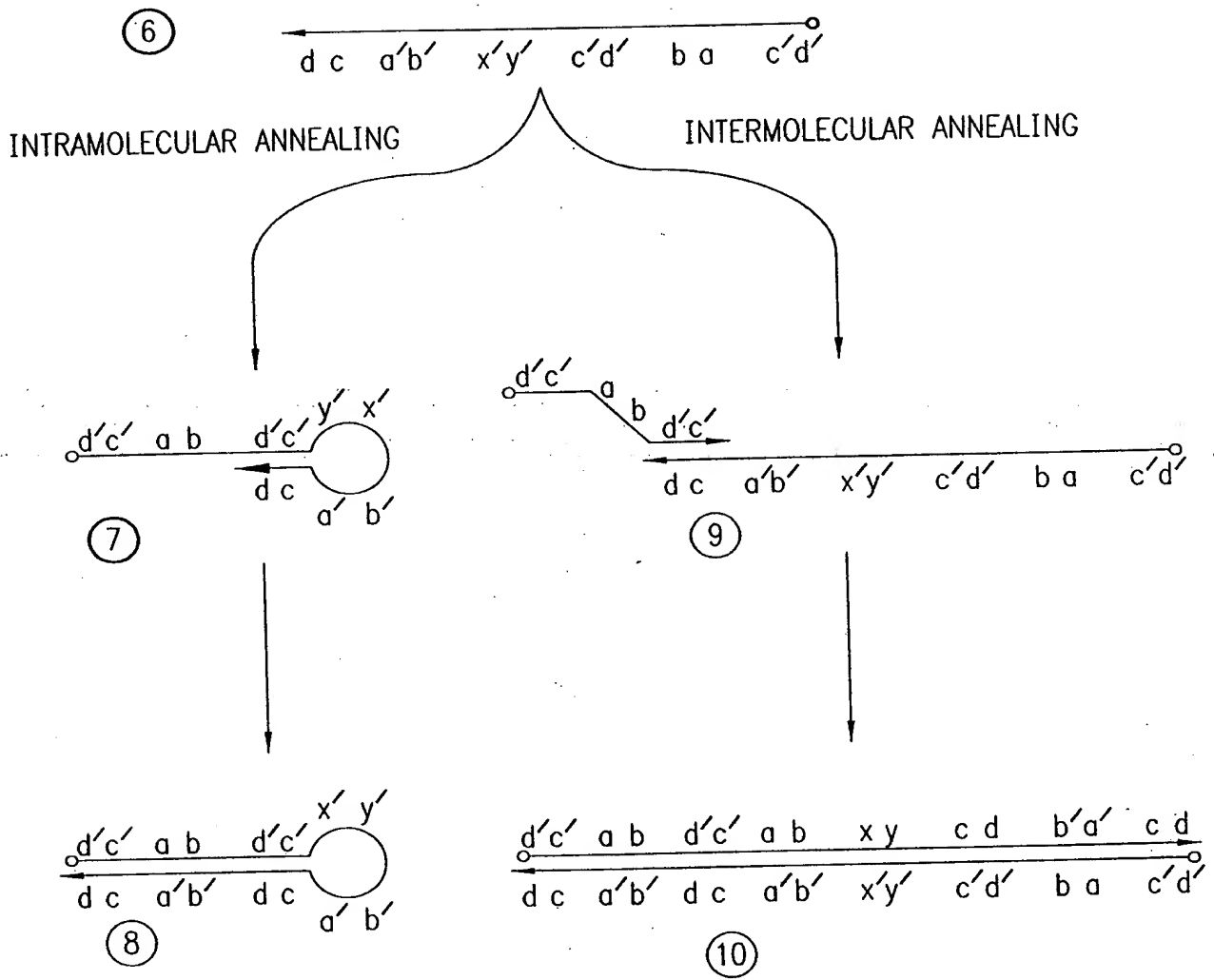


FIG. 10

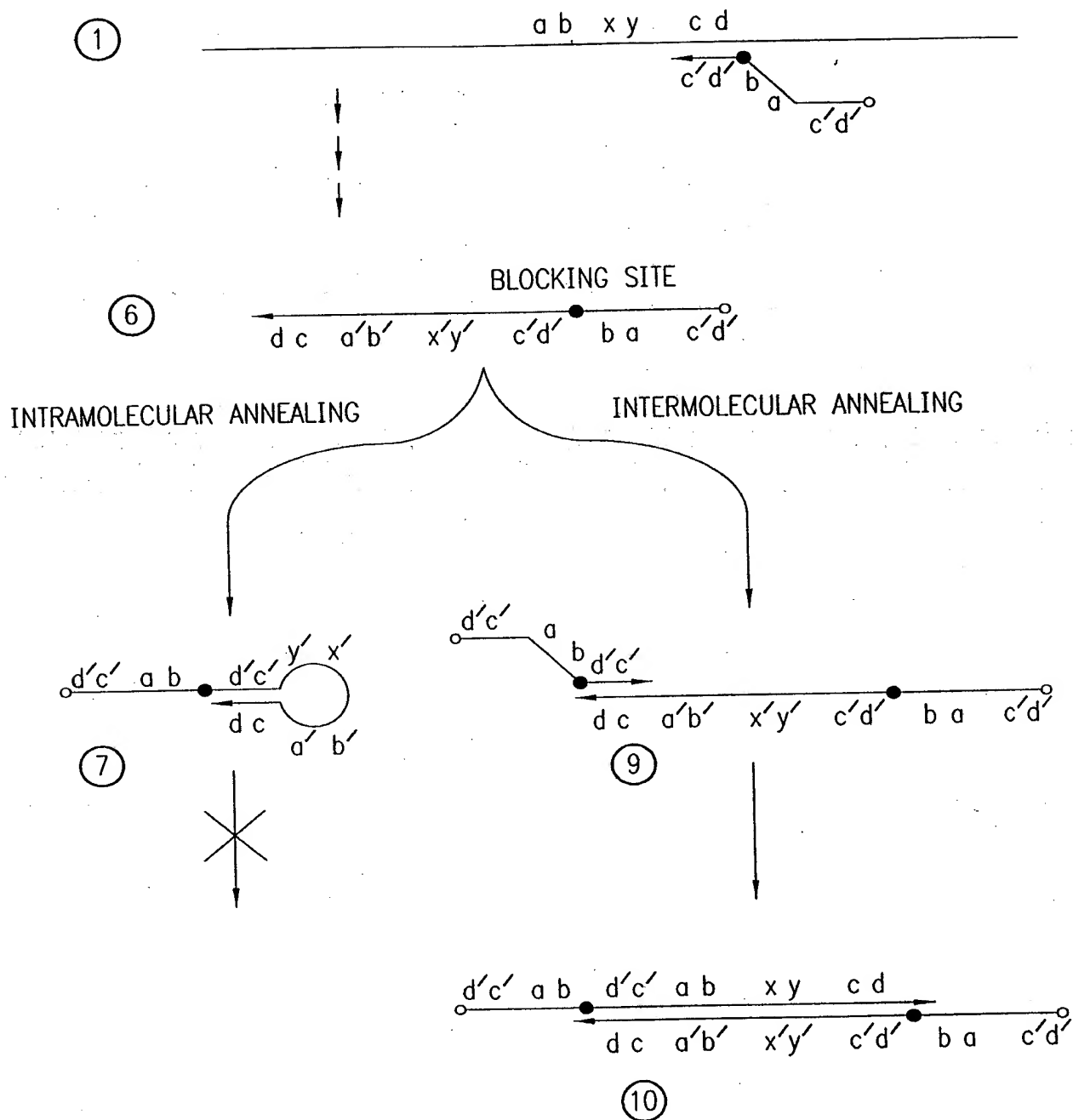


FIG. 11

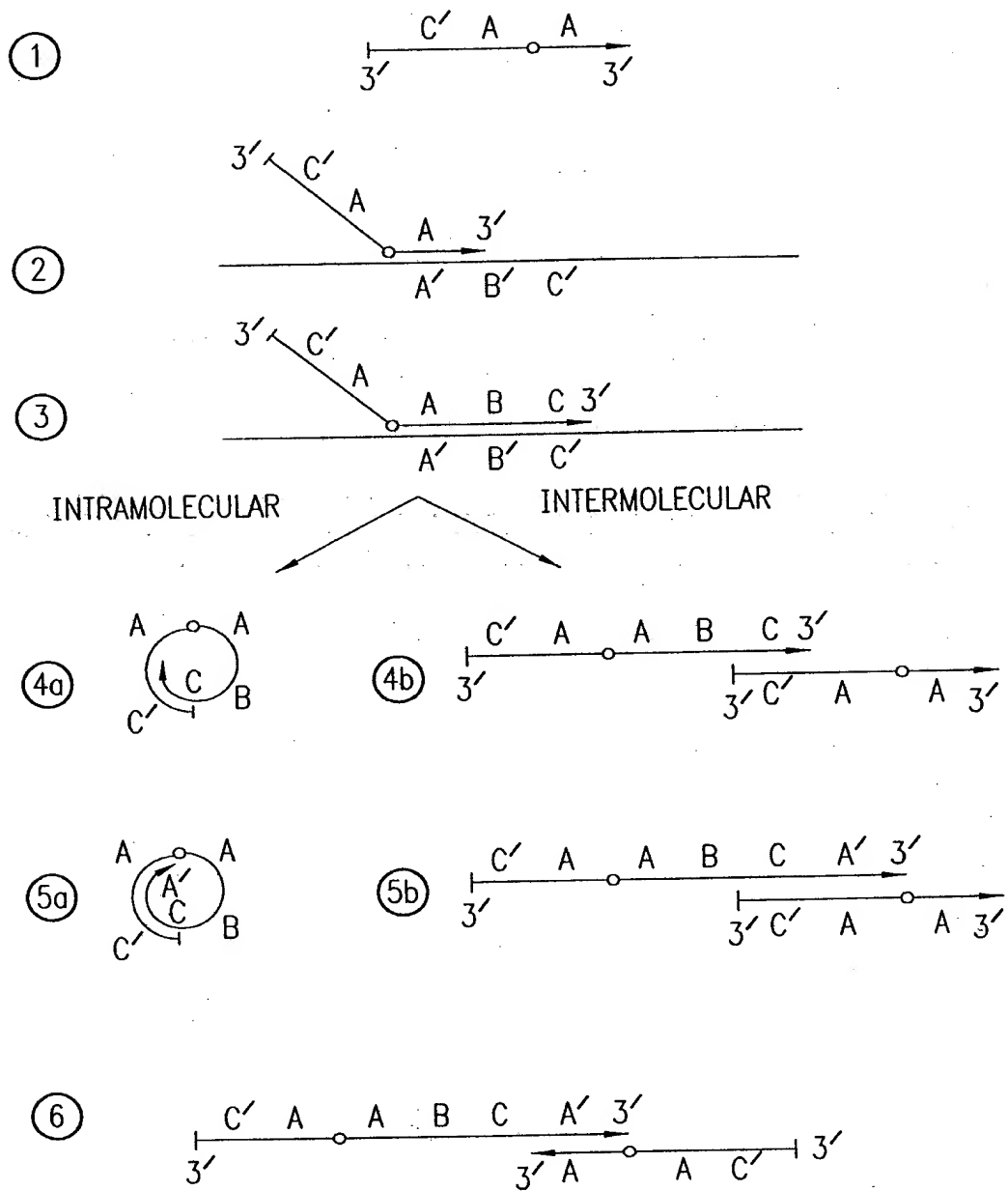


FIG. 12

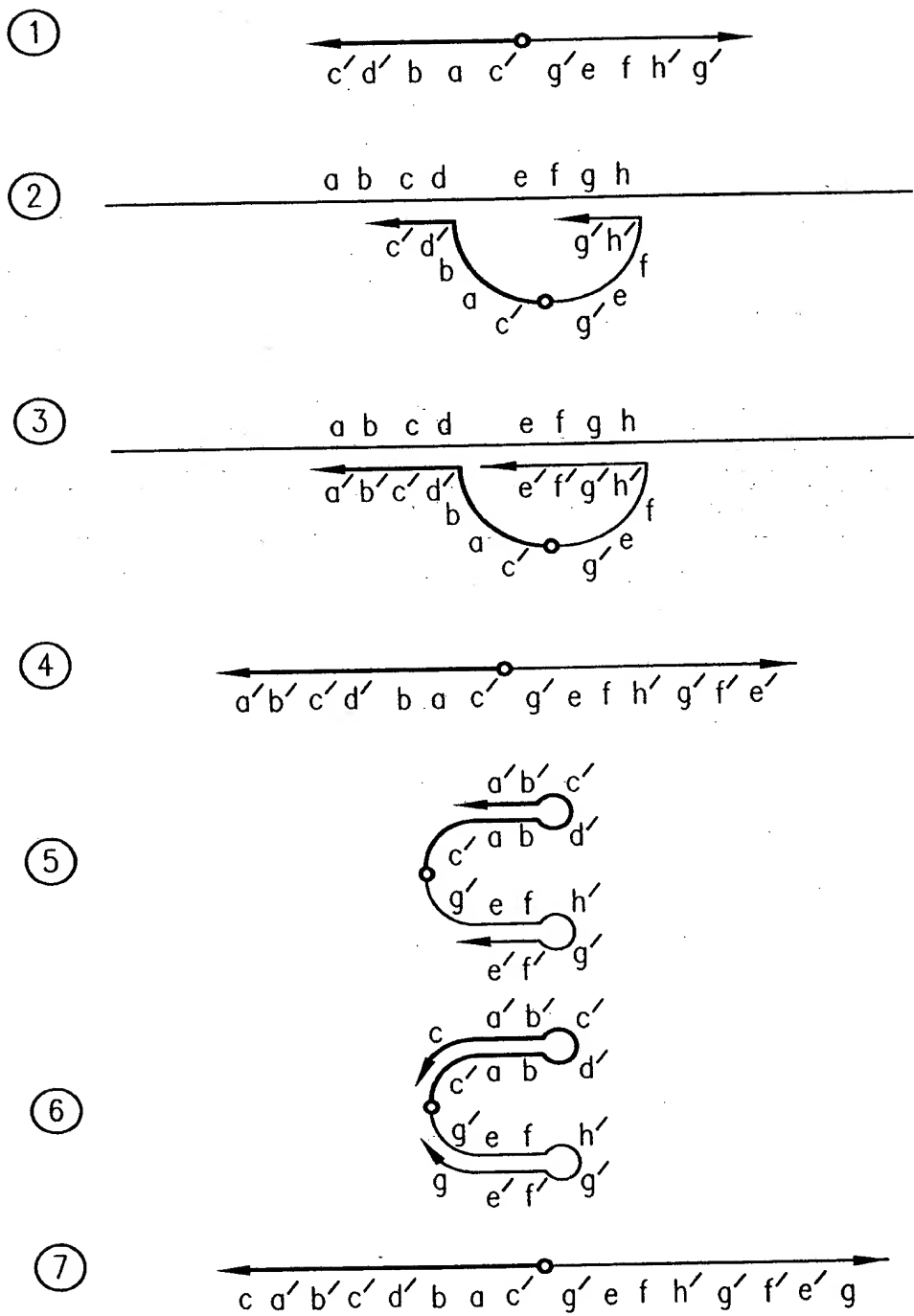


FIG. 13

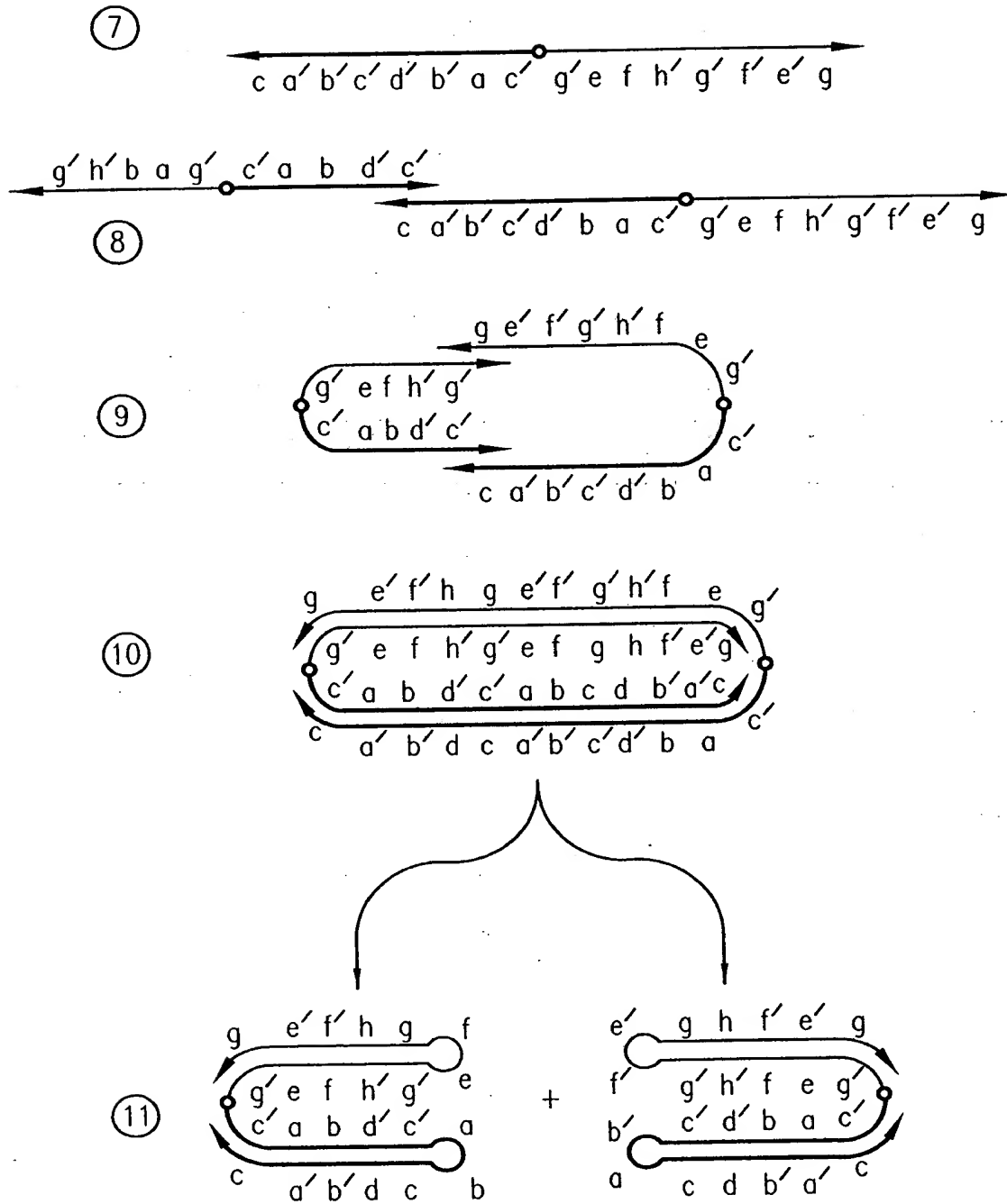
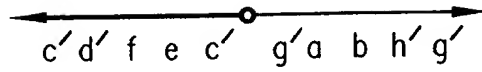
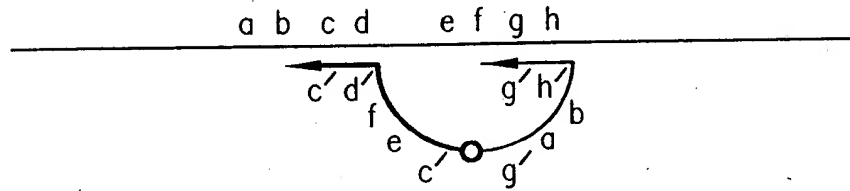


FIG. 14

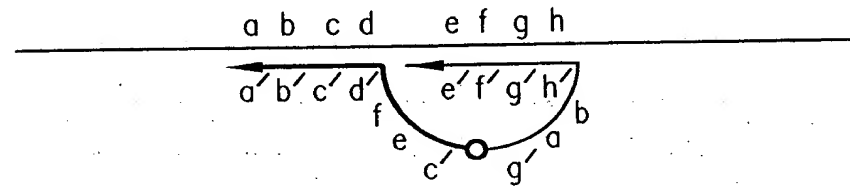
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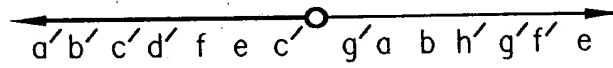
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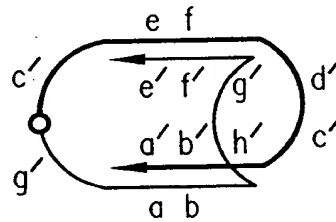
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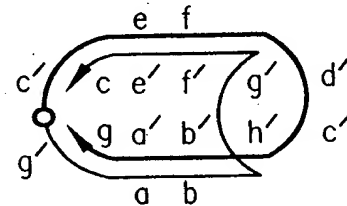
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⑤



⑥



FORM I

⑦

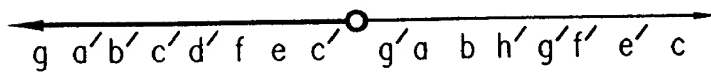
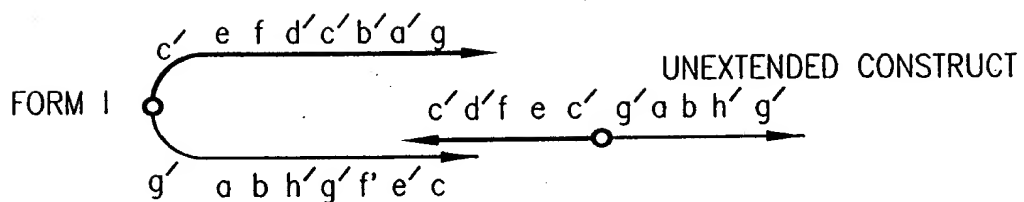
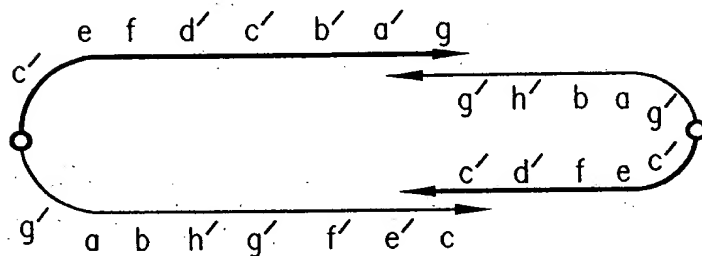


FIG. 15

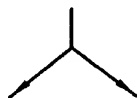
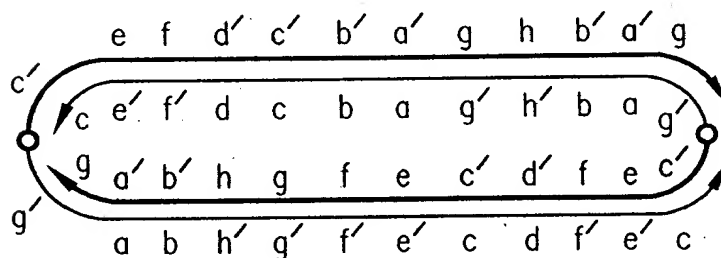
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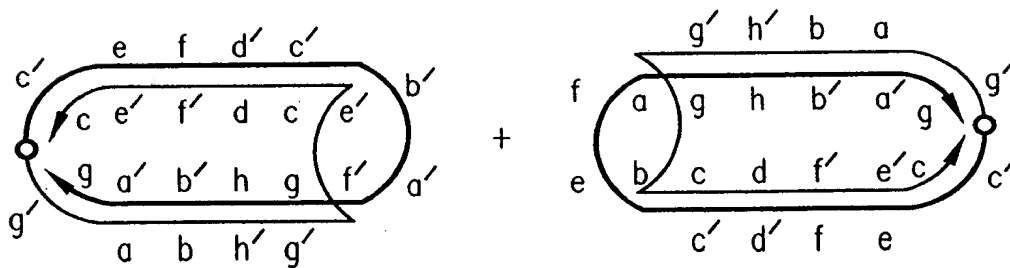
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③



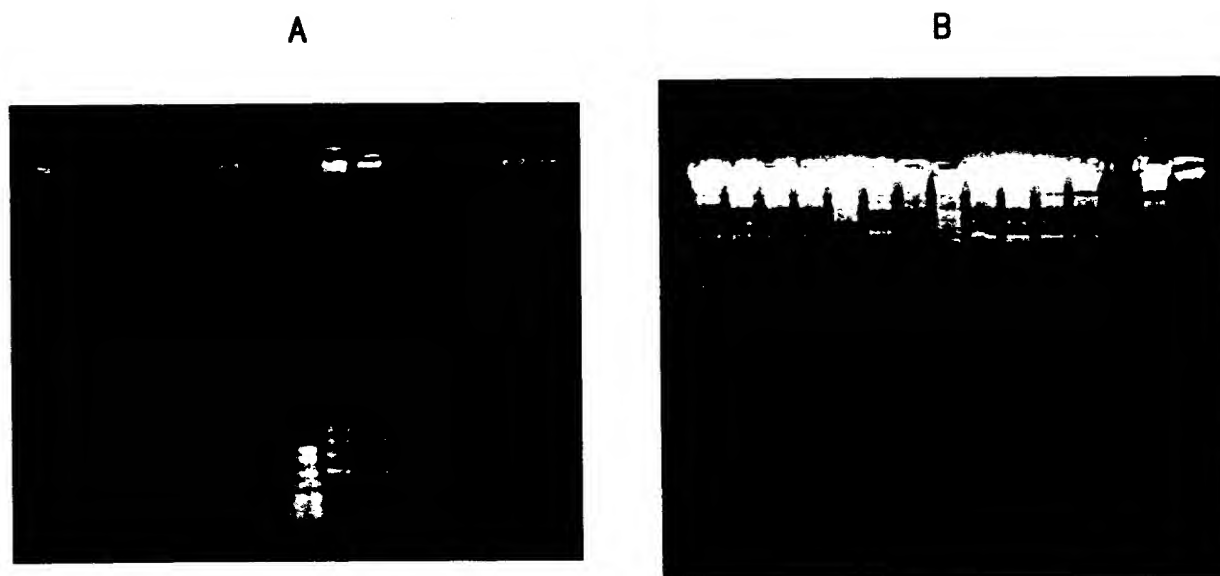
④



FORM II

FORM III

FIG. 16



30 MINUTES INCUBATION

180 MINUTES INCUBATION

- 1 53°C, 10<sup>-2</sup> DILUTION
- 2 53°C, 10<sup>-3</sup> DILUTION
- 3 53°C, 10<sup>-4</sup> DILUTION
- 4 53°C, 10<sup>-5</sup> DILUTION
- 5 53°C, NO TARGET
- 6 53°C, 10<sup>-2</sup> DILUTION, FC/LRC
- 7 53°C, 10<sup>-2</sup> DILUTION, LFC/RC
- 8 MSP I MARKER
- 9 63°C, 10<sup>-2</sup> DILUTION
- 10 63°C, 10<sup>-3</sup> DILUTION
- 11 63°C, 10<sup>-4</sup> DILUTION
- 12 63°C, 10<sup>-5</sup> DILUTION
- 13 63°C, NO TARGET
- 14 63°C, 10<sup>-2</sup> DILUTION, FC/LRC
- 15 63°C, 10<sup>-2</sup> DILUTION, LFC/RC

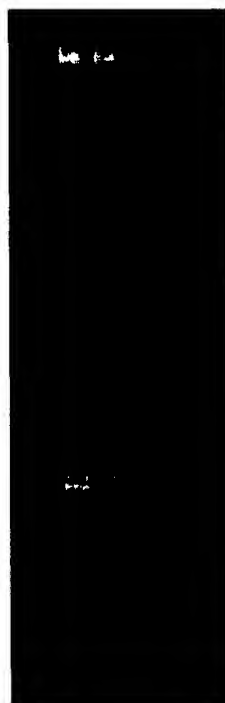
FIG. 17

A) GEL ASSAY

TOP = ISOTHERMAL AMPLIFICATION

BOTTOM = PCR AMPLIFICATION

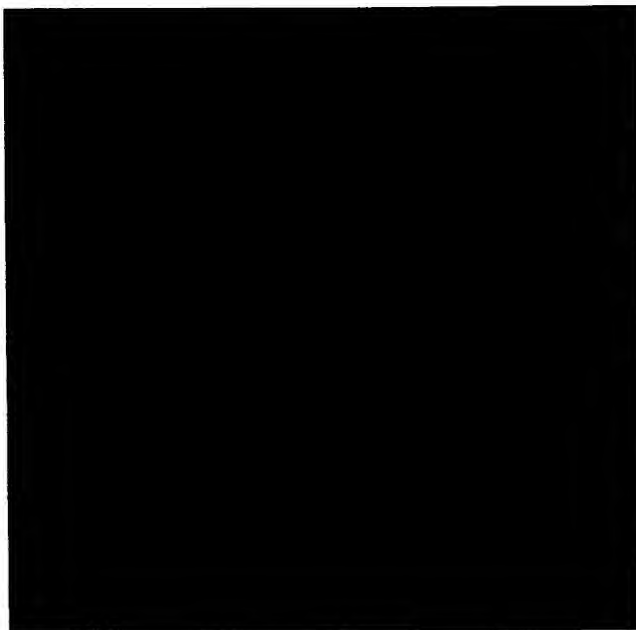
- 1 MSP | MARKER
- 2  $1 \times 10^6$  TARGET
- 3  $1 \times 10^4$  TARGET
- 4  $1 \times 10^2$  TARGET
- 5 NO TARGET



B) PLATE ASSAY

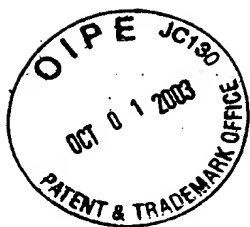
$10^6$ TARGET	$10^4$ TARGET	$10^2$ TARGET	TARGET
1.702	1.594	0.376	0.085

FIG. 18



- 1 CARBOXY-U, KLENOW 37°C, NEB #2
- 2 NORMAL T, KLENOW, 37°C, NEB #2
- 3 CARBOXY-U, KLENOW, 37°C, BUFFER #2A
- 4 NORMAL T, KLENOW, 37°C, BUFFER #2A
- 5 CARBOXY-U, KLENOW, 55°C, NEB #2
- 6 NORMAL T, KLENOW, 55°C, NEB #2
- 7 MSP I MARKER
- 8 CARBOXY-U, TAQ, 55°C, NEB #2
- 9 NORMAL T, TAQ, 55°C, NEB #2
- 10 CARBOXY-U, TAQ, 65°C, BUFFER #2M
- 11 NORMAL T, TAQ, 65°C, BUFFER #2M
- 12 CARBOXY-U, BST, 65°C, THERMOPOL BUFFER
- 13 NORMAL T, BST, 65°C, THERMOPOL BUFFER
- 14 CARBOXY-U, TAQ, 65°C, BUFFER #2A
- 15 NORMAL T, TAQ, 65°C, BUFFER #2A

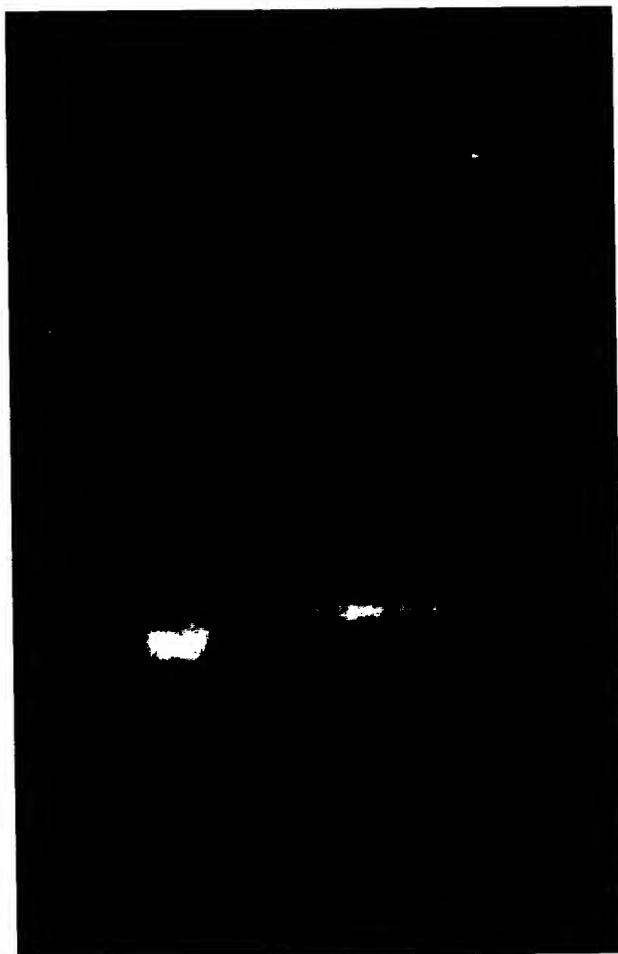
FIG. 19



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ENZYME	BUFFER	TEMPERATURE	NUCLEOTIDE	RELATIVE LEVEL OF SYNTHESIS
KLENOW	NEB #2	37°C	CARBOXY U	+
			NORMAL T	+++
KLENOW	2A	37°C	CARBOXY U	-
			NORMAL T	++
KLENOW	NEB #2	55°C	CARBOXY U	+
			NORMAL T	+++
TAQ	NEB #2	55°C	CARBOXY U	++
			NORMAL T	++++
TAQ	2M	65°C	CARBOXY U	++
			NORMAL T	++++
BST	THERMOPOL	65°C	CARBOXY U	++
			NORMAL T	++++
TAQ	2A	65°C	CARBOXY U	+/-
			NORMAL T	+++

FIG. 20

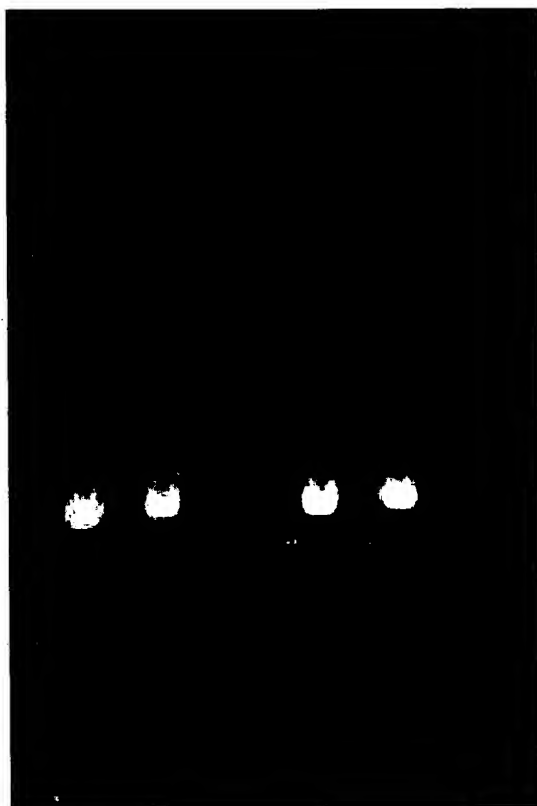


1. MSP I/BST E II MARKER
2. NORMAL T, 1 mM  $MgCl_2$
3. CARBOXY U, 2 mM  $MgCl_2$
4. CARBOXY U, 3 mM  $MgCl_2$
5. CARBOXY U, 4 mM  $MgCl_2$
6. CARBOXY U, 5 mM  $MgCl_2$
7. MSP I/BST E II MARKER

FIG. 21

02570 U.S. PTO  
10/01/03

22/30

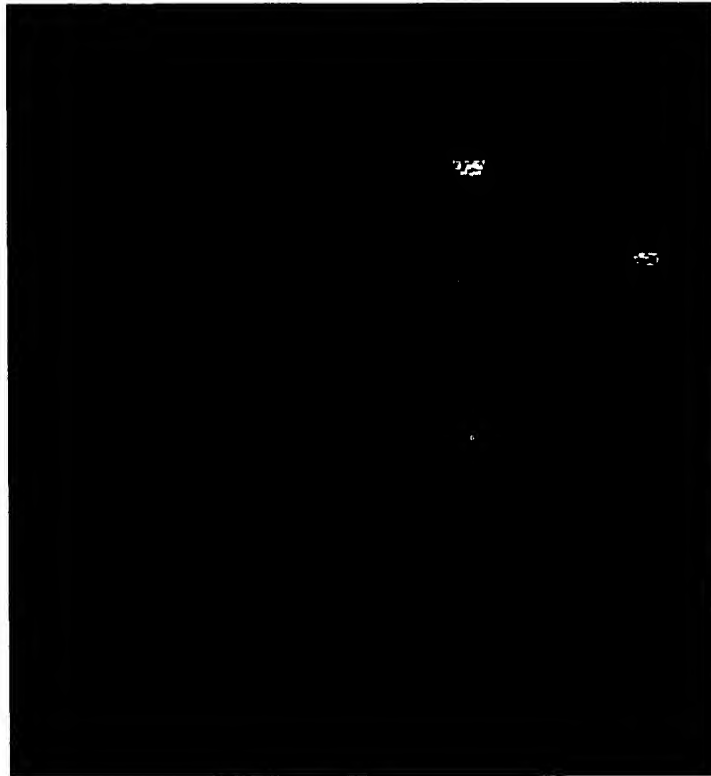


1. MSP I/BST E II MARKER
2. NORMAL T, TAQ
3. CARBOXY U, TAQ
4. NORMAL T, Tfi
5. CARBOXY U, Tfi
6. NORMAL T, Tth
7. CARBOXY U, Tth
8. NORMAL T, AMPLITHERM
9. CARBOXY U, AMPLITHERM
10. NORMAL T, REPLITHERM
11. CARBOXY U, REPLITHERM
12. MSP I/BST E II MARKER

FIG. 22

02570 U.S. PTO  
10/01/03

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1. TAQ, 2mM  $\text{MgCl}_2$
2. TAQ, 4mM  $\text{MgCl}_2$
3. TAQ, 6mM  $\text{MgCl}_2$
4. Tfl, 2mM  $\text{MgCl}_2$
5. Tfl, 4mM  $\text{MgCl}_2$
6. Tfl, 6mM  $\text{MgCl}_2$
7. MSP I MARKER
8. Tfl/Enh, 2mM  $\text{MgCl}_2$
9. Tfl/Enh, 4mM  $\text{MgCl}_2$
10. Tfl/Enh, 6mM  $\text{MgCl}_2$

FIG. 23

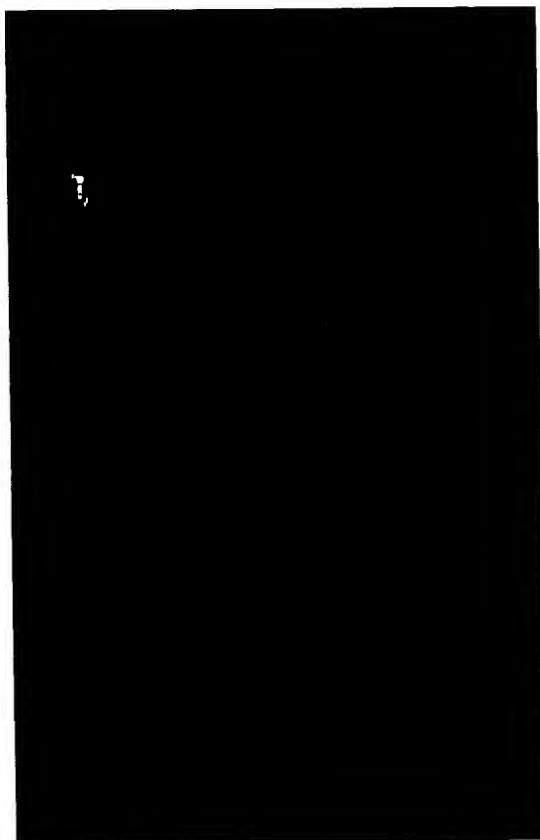


1. Tth/Enh, 4mM  $\text{MgCl}_2$
2. Tth/Enh, 6mM  $\text{MgCl}_2$
3. Tth/Enh, 8mM  $\text{MgCl}_2$
4. Msp I/BspE1 MARKER
5. AMPLITHERM/ Enh, 4mM  $\text{MgCl}_2$
6. AMPLITHERM/ Enh, 6mM  $\text{MgCl}_2$
7. AMPLITHERM/ Enh, 8mM  $\text{MgCl}_2$
8. Msp I/BspE1 MARKER
9. REPLITHERM/ Enh, 4mM  $\text{MgCl}_2$
10. REPLITHERM/ Enh, 6mM  $\text{MgCl}_2$
11. REPLITHERM/ Enh, 8mM  $\text{MgCl}_2$

FIG. 24

02570 U.S. PTO  
10/01/03

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1. Msp I MARKER
2. 0.3X ENHANCER
3. CONTROL
4. DEAZA G
5. GENE 32
6. 10% DMSO
7. 3X POLYMERASE

*FIG. 25*



SEQ ID 11

5' -TGC GCT GCT AAC AAA GCC CGA AAG GAA G-----GCT GAA AGG AGG AAC TAT ATG GCG TCA TAC GAT ATG AAC GTT-3'  
3' -ACG CCA CCA TTG TTT CGG GCT TTC CTT C-----CGA CTT TCC TCC TTG ATA TAC GCG AGT ATG CTA TAC TTG CAA-5'

SEQ ID 12

TS-13 SEQ ID 13

5' -AAT CTA GA GCT AAC AAA GCC CGA AAG GAA G-3'

SEQ ID 16 TS-14

3' -CGA CTT ICC ICC TTG ATA TA GAC GTC TT-5'

TS-21 SEQ ID 14

5' -TGC GCT GCT AAC AAA GCC CGA AAG GAA G-3'

SEQ ID 17 TS-23

3' -CGA CTT ICC ICC TTG ATA TAC GCG AGT -5'

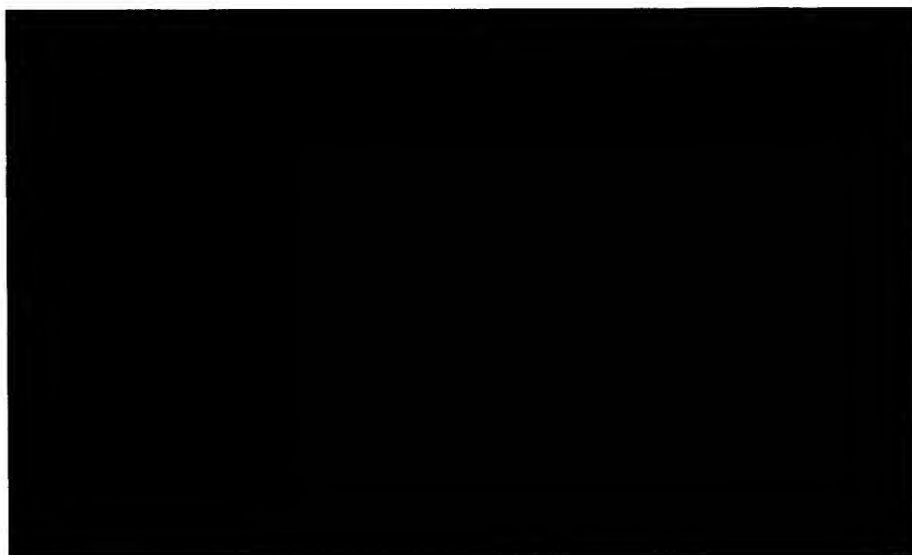
TS-22 SEQ ID 15

5' -ACC CGC GCT GCT AAC AAA GCC CGA AAG GAA G-3'

SEQ ID 18 TS-24

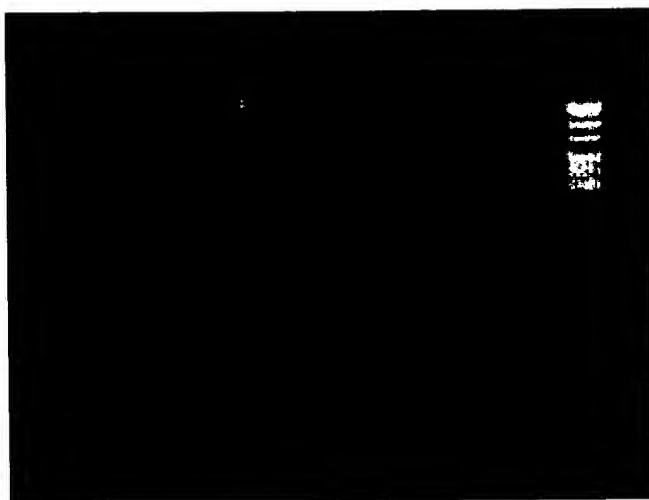
3' -G ATA TAC GCG AGT ATG CTA TAC TTG CAA-5'

FIG. 26



1. Msp I MARKER
2. TS13 + TS14
3. TS13 + TS23
4. TS13 + TS24
5. TS21 + TS14
6. TS21 + TS23
7. TS21 + TS24
8. TS22 + TS14
9. TS22 + TS23
10. TS22 + TS24
11. Msp I MARKER
12. TS13 + TS14 (DIFFERNT LOT OF C-U)
13. TS13 + TS14 (ALLYLAMINE dUTP)
14. TS13 + TS14 (NORMAL dTTP)

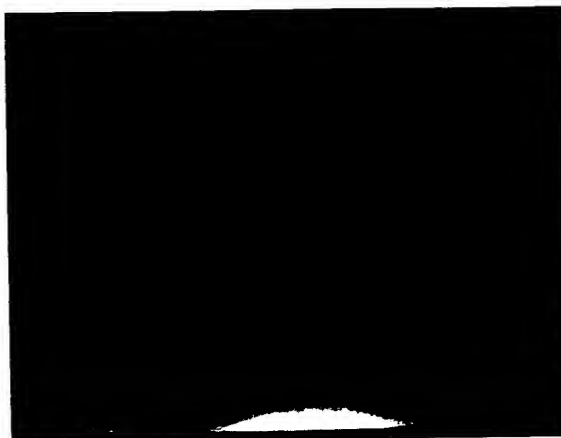
FIG. 27



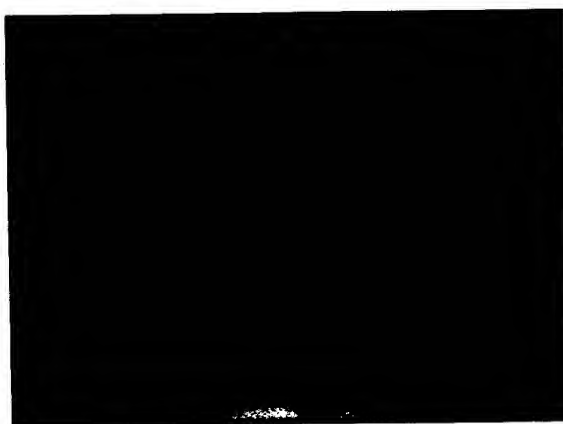
1. TS13 + TS14
2. TS13 + TS23
3. TS13 + TS24
4. Msp | MARKER
5. TS21 + TS14
6. TS21 + TS23
7. TS21 + TS24
8. TS22 + TS14
9. TS22 + TS23
10. TS22 + TS24
11. Msp | MARKER

*FIG. 28*

FLOURESCENT DETECTION



ETHIDIUM BROMIDE FLOURESCENCE



- 1 1 x TAPS, pH 9.2
- 2 2 x TAPS, pH 9.2
- 3 3 x TAPS, pH 9.2
- 4 3 x TAPS, pH 9.7
- 5 3 x TAPS, pH 9.2
- 6 3 x TAPS, pH 8.6
- 7 NO ENZYME CONTROL
- 8 FLUORESCCEIN 12-ddUTP CONTROL

FIG. 29

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FLOURESCENT DETECTION



ETHIDIUM BROMIDE FLOURESCENCE



- 1 1 x TAPS, pH 9.2
- 2 2 x TAPS, pH 9.2
- 3 3 x TAPS, pH 9.2
- 4 3 x TAPS, pH 9.7
- 5 3 x TAPS, pH 9.2
- 6 3 x TAPS, pH 8.6
- 7 NO ENZYME CONTROL
- 8 FLUORESCCEIN 12-ddUTP CONTROL

FIG. 30